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Removal of Antibiotics in Biological Wastewater Treatment Systems—A Critical Assessment Using the Activated Sludge Modeling Framework for Xenobiotics (ASM-X)

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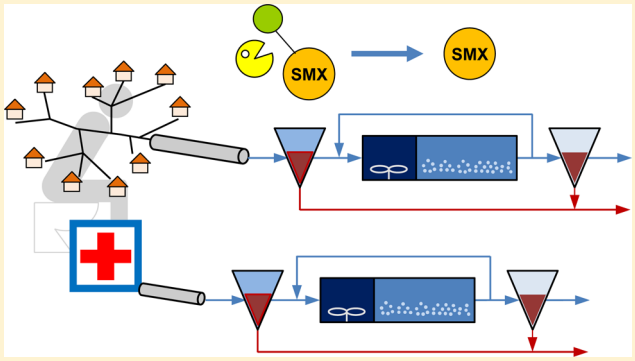
Supporting Information

ABSTRACT: Many scientific studies present removal efficiencies for pharmaceuticals in laboratory-, pilot-, and full-scale wastewater treatment plants, based on observations that may be impacted by theoretical and methodological approaches used. In this Critical Review, we evaluated factors influencing observed removal efficiencies of three antibiotics (sulfamethoxazole, ciprofloxacin, tetracycline) in pilot- and full-scale biological treatment systems. Factors assessed include (i) retransformation to parent pharmaceuticals from e.g., conjugated metabolites and analogues, (ii) solid retention time (SRT), (iii) fractions sorbed onto solids, and (iv) dynamics in influent and effluent loading. A recently developed methodology was used, relying on the comparison of removal efficiency predictions (obtained with the Activated Sludge Model for Xenobiotics (ASM-X)) with representative measured data from literature. By applying this methodology, we demonstrated that (a) the elimination of sulfamethoxazole may be significantly underestimated when not considering retransformation from conjugated metabolites, depending on the type (urban or hospital) and size of upstream catchments; (b) operation at extended SRT may enhance antibiotic removal, as shown for sulfamethoxazole; (c) not accounting for fractions sorbed in influent and effluent solids may cause slight underestimation of ciprofloxacin removal efficiency. Using tetracycline as example substance, we ultimately evaluated implications of effluent dynamics and retransformation on environmental exposure and risk prediction.



INTRODUCTION

Biological wastewater treatment plants (WWTPs) have a crucial role in the mitigation of the risk posed by the release of pharmaceuticals in receiving environmental bodies. The elimination of pharmaceuticals in conventional WWTPs is presently considered insufficient, and a number of substances of concern for the aquatic environment have been identified at national[1,2] and regional levels.[3] Among existing pharmaceutical classes, research has focused on antibiotics because of their ubiquitous usage, their overall recalcitrance to biological wastewater treatment, and more recently, antibiotic resistance spread by WWTP emissions.[4−7]

Because of the comparably high costs and the uncertainty inherent in the analysis of pharmaceuticals, simulation models represent an appealing option to investigate their fate and elimination in biological WWTPs. Two recent review articles[8,9] summarized the approaches used for fate prediction of xenobiotic trace chemicals in wastewater, including pharmaceuticals. Nevertheless, adequate assessment of pharmaceutical elimination in biological WWTPs still remains a challenge, considering the high variability of measured removal efficiencies presented in literature for the same substance.[6,10−13] Thus, combining modeling and experimental/analytical efforts offers the opportunity for a thorough understanding of elimination mechanisms in WWTPs. It also represents a valuable option for decision support to operators and legislators, given the progressive implementation of WWTP upgrade measures to reduce emissions of trace chemicals.[14−17]

In the last three decades, ever since pharmaceuticals were detected in aqueous media, several review articles on the fate of pharmaceuticals and, more specifically, antibiotics during wastewater treatment have been published.[5,6,10,12,18−31] The main objective of this study was to complement previous assessments by critically evaluating factors influencing the elimination of antibiotics and by testing a model-based methodology to identify the influence of such factors. The assessment focused on the influence of (i) retransformation to parent antibiotics via, for example, metabolite deconjugation, (ii) solid retention time (SRT) in secondary treatment, (iii) fractions sorbed onto influent and effluent solids, and (iv) intrinsic dynamics in influent and effluent loading. The developed

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methodology relies on the generalization of the calibrated Activated Sludge Modeling Framework for Xenobiotics (ASM-X[32−34]) with full-scale international removal efficiency data thoroughly selected from peer reviewed literature. The methodology, already tested for diclofenac and carbamazepine,[33] was further considered for three antibiotics (sulfamethoxazole, SMX; ciprofloxacin, CIP; tetracycline, TCY), for which the model had already been calibrated and evaluated with batch and full-scale experimental data, respectively.[32] An overview of the therapeutic use of the selected antibiotics is provided in the Supporting Information.

FACTORS AND PROCESSES INFLUENCING ANTIBIOTIC REMOVAL IN WWTPS

Retransformation Processes. In the following subsections, we assess in detail a number of processes responsible for "negative" removal efficiencies[35] observed in full-scale WWTPs for parent pharmaceuticals. A critical overview of processes, not traditionally accounted for when assessing pharmaceutical fate, is provided to propose a more integrated approach to fate assessment.

Deconjugation of Metabolites. The human metabolism of administered pharmaceuticals involves two types of mechanisms, that is, functionalization reactions (e.g., oxidation, hydroxylation, reduction, mostly catalyzed by CYP450 enzymes) and conjugation reactions (e.g., glucuronidation, sulfation, acetylation). The latter mechanisms increase the polarity of organic molecules to facilitate their excretion. The traditional distinction of these reactions as Phase I and Phase II metabolism, respectively, has been lately questioned, as direct conjugation of parent pharmaceuticals frequently occurs.[36,37] Conjugated metabolites of parent pharmaceuticals can undergo retransformation back to the parent form following cleavage of the conjugated moiety. Importantly, this type of conjugates can represent up to one-quarter (22%) of first-step pharmaceutical metabolites,[37] indicating a potential for the formation of parent forms after excretion.

Microbial deconjugation of human metabolites to parent forms has been hypothesized or observed in wastewater for estrogens,[38−40] carbamazepine,[33,41−44] and its functionalized metabolites[44−46] and diclofenac.[33,47,48] For the latter two substances, deconjugation was considered to explain concentration increases from WWTP influent to effluent. Although the significance of deconjugation during wastewater treatment has been acknowledged, detailed empirical evidence is still scarce, being limited to estrogens, because of analytical challenges.[49−52]

With respect to the antibiotics considered in this study, the metabolism has been widely investigated, allowing for the identification of potentially retransformable excreted forms. A detailed description of the metabolism and excretion of SMX, CIP and TCY and their metabolites is presented in the Supporting Information (Figures S1−S4 and Table S1). The human metabolism of SMX involves the excretion of acetylated (N_4-acetyl-SMX) and glucuronide (SMX-N_1-Glu, SMX-2′-Glu) conjugates of the parent substance, which combined in urine account from 50% up to 75% of an administered dose of SMX (Figure S4 and Table S1).[53,54] Extensive literature exists on the deconjugation of the major metabolite, N_4-acetyl-SMX, during wastewater treatment.[55−59] Retransformation of acetylated metabolites back to parent forms has also been observed for other sulfonamides in aqueous media.[60] More recently, SMX-N_1-Glu was detected in preclarified[61] and final WWTP effluent[62] and receiving surface water bodies,[63] possibly indicating its persistence (as for other N-glucuronide pharmaceutical metabolites[44]). Conversely, almost complete elimination (>90%) was observed during secondary treatment[61] and comparably fast microbial deconjugation of SMX-N_1-Glu occurred in water-sediment tests.[64] Excreted conjugated metabolites of CIP include sulfo-CIP and N-formyl-CIP, the former being the main conjugated metabolite (up to 17% of an administered dose).[65,66] Minor excretion of the glucuronide conjugate (CIP-Glu) has also been reported,[67] although this metabolite has never been identified in wastewater. To the authors' knowledge, microbial deconjugation of sulfo-CIP in aqueous media has not been investigated, but can be postulated in analogy with other sulfate metabolites (e.g., of natural and synthetic estrogens[40,68,69]). Orally and intravenously administered TCY undergoes limited metabolism in humans, being excreted mainly in unchanged form (Figure S1).[70−72] No formation of conjugated metabolites has been reported.

Abiotic Retransformation of Metabolites and Transformation Products. Minor excretion (5%) of TCY in the form of the optical isomer 4-epi-tetracycline (4-epi-TCY) has been reported.[73,74] 4-epi-TCY has been also identified as abiotic transformation product of TCY in aqueous solution,[75] activated sludge,[76] and manure.[77−79] In liquid media, epimerization of TCY was shown to be reversible, mostly occurring under acidic and neutral conditions.[77,80] Epimerization is catalyzed by the presence of anions,[80] whereas reverse epimerization is facilitated in the presence of divalent cations at pH > 6.[81,82] Up to 70% abiotic retransformation of 4-epimers of TCY and its analogues was shown in soil and activated sludge at pH = 8.1 and in the presence of magnesium and calcium ions.[76] Reversible epimerization, with formation of TCY from 4-epi-TCY, is thus identifiable as abiotic retransformation process. 4-epi-TCY concentration was quantified in two hospital effluents at $\mu g\ L^{-1}$ levels,[83] being 11% and 35% of corresponding TCY concentration. Comparable concentrations of TCY and 4-epi-TCY (~100 ng L^{-1}) were also measured in the influent of a municipal WWTP, where almost complete removal of the two substances was reported to occur via sorption onto activated sludge.[84]

Nitration and nitrosation are common abiotic transformation mechanisms for trace chemicals containing aromatic groups.[85] The formation of nitrated products was shown in pure and mixed nitrifying cultures for the estrogen 17α-ethinyl estradiol (EE2)[86−89] and acetaminophen,[90] being associated with significant accumulation of nitrite or peroxynitrite and acidic pH conditions. Nitrated and nitrosated derivatives were further identified and quantified (at low ng L^{-1} levels) in full-scale WWTPs for SMX[91] and diclofenac.[91−93] Although nitration and nitrosation appear of limited importance during conventional treatment of domestic wastewater,[85−89] these mechanisms may be relevant to the treatment of high strength reject water and/or in deammonification and nitrite shunt processes, where nitrite accumulation is more likely to occur. Notably, parent SMX was shown to be abiotically formed from its nitrosated (via photolysis)[63] and nitrated[94] derivatives.

Formation from Analogues and Structurally Related Chemicals. Pharmaceuticals and their human metabolites can be additionally formed from other commercial chemicals (a) as a result of human metabolism and (b) as products of biological transformation processes during, for example, wastewater treatment. Formation typically occurs from substances exhibiting structural similarity to the formed pharmaceutical. Examples are oxazepam, a benzodiazepine pharmaceutical and a human metabolite of the analogues diazepam and temazepam,

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and atenolol acid, a human metabolite of metoprolol and a transformation product of atenolol.[95−97] Formation from other commercial chemicals via metabolism (following veterinary use) and environmental biodegradation have been reported for CIP and TCY. CIP was observed to be a metabolite (up to 47% of the administered dose[98−100]), fungal degradation product,[101] and photolysis product[102] of enrofloxacin. Rolitetracycline, a tetracycline analogue used for veterinary therapy, can be metabolized to TCY.[103]

Release from Faecal Matter. Concentration increases between the influent and the effluent of a primary clarifier were reported for fluoroquinolones, including CIP,[104,105] and macrolides,[56] possibly resulting from the release from faeces to the aqueous phase.[56] Similar observations were made for macrolides by comparing full-scale WWTP influents and effluents.[106−108] Although significant macrolide amounts have been detected in raw influent solids,[59] alternative hypotheses were made to explain macrolide formation.[109] Release from faecal matter is relevant to CIP and TCY, considering the substantial excretion of these antibiotics in faeces (9%−52%, Table S1).[66,72]

Hydrolysis of Particulate and Colloidal Matter. Particulate matter in biological wastewater treatment includes microbial cells, bacterial decay products, exocellular polymeric substances, hydrolyzable and colloidal organic matter and inorganic material.[110−115] Hydrolyzable and colloidal organics undergo hydrolysis by extracellular enzymes,[116,117] with potential release of sorbed trace chemicals to the aqueous phase. Sorption onto colloidal matter in activated sludge was found significant for hydrophobic chemicals (e.g., polycyclic aromatic hydrocarbons).[111−113] As for pharmaceuticals, little information is available on the sorption onto different matrices. In activated sludge, partitioning of 17α-ethinylestradiol to dissolved and colloidal matter was found to be negligible compared to sludge particles.[118] Nevertheless, partitioning of CIP and TCY to organic constituents other than particulates can be expected relevant, as previously shown for municipal biosolids[119] or purified humic acids.[120]

Desorption. CIP and TCY are multivalent zwitterions with strong dipole, having high water solubility and comparably low logD. Nevertheless, both chemicals exhibit significant sorption capacity onto suspended solids and sludge.[121−124] Sorption of these chemicals mainly occurs via hydrophobicity-independent mechanisms (e.g., electrostatic attraction, cation bridging).[119,125−131] Sorption equilibria for CIP are strongly influenced by pH and ionic strength, and can change under varying conditions in biological wastewater treatment.[124] A similar behavior can be postulated for TCY, based on previous observations of pH-dependent sorption in soils.[128] Further observations[33,122,132,133] showed that significant fractions of sorbed pharmaceuticals can be sequestered in the sludge matrix, undergoing slow or no desorption (sorption hysteresis[132]) and thus not being in equilibrium with the aqueous phase.

Solid Retention Time (SRT). The influence of the solid retention time (SRT) on the elimination of pharmaceuticals has been the object of intense investigation in the past decade. Macroscopically, SRT-related effects were observed in terms of increased pharmaceutical transformation: (i) in the presence of nitrifiers (supported at SRT greater than 5 d in fully aerobic systems and 8−10 d in aerobic-anoxic systems[134]), as compared to heterotrophs only;[89,135−149] and (ii) in systems with extended physical retention of microbial biomass and thus operating at prolonged SRTs (e.g., membrane bioreactors—MBRs, biofilm reactors).[10,26,33,39,57,58,150−162] In both cases, the influence of SRT could be explained by changes induced in microbial communities, and two hypotheses have been considered to summarize such benefits.

On the one hand, SRT has a positive effect on the microbial diversity by inducing an expansion of the microbial community. This has been demonstrated in laboratory-[163] and full-scale[155] activated sludge systems. Increased biodiversity may stem from the enrichment of slow-growing bacteria[21] (e.g., nitrifiers), specialist degrader strains[155,164,165] responsible for specific transformation processes[166] and K-strategists, capable of efficiently utilizing resources at low concentrations.[153,155] This may translate into an enhancement of the overall biotransformation potential of the community. On the other hand, at prolonged SRT biomass is increasingly exposed to limiting substrate availability conditions, resulting from operation at reduced food-to-microorganism (F/M) ratios. Under oligotrophic conditions, the microbial metabolism of heterotrophic bacteria relies on fortuitous oxidation (resulting from low enzyme specificity)[167,168] or the utilization of multiple substrates at low concentration (mixed substrate growth).[169,170] Through broad expression of enzymes responsible of catabolism, bacteria can utilize organic substances not typically used as growth substrates[169−172] (a strategy also referred to as metabolic expansion).[21]

In mixed culture systems, extended SRT results in the enrichment of slow-growing nitrifiers and the concomitant exposure of heterotrophs to growth substrate limiting conditions, both likely favorable toward trace chemical biotransformation. For the estrogen EE2, heterotrophic bacteria have been found to biodegrade transformation products generated by ammonia oxidizers, thereby demonstrating an example of cooperative contribution toward the removal of trace chemicals.[89]

To date, evidence of the influence of SRT on biotransformation capacity has been disputed or could not be generalized for all pharmaceuticals investigated,[39,42,57,58,87,89,136,139,143,145−148,150,156,158,160,173−178] leading to the conclusion that the impact of SRT may be chemical-specific. In a number of cases, controversial results have been obtained for the same substance (e.g., trimethoprim,[89,137] atenolol[145,173]), possibly indicating the interference of experimental and methodological approaches used. Among other sources of variability, growth substrate availability (abundance or limitation), growth conditions (batch or chemostat), and the resulting physiological state of biomass may have influenced observations of trimethoprim biotransformation by nitrifiers.[86,89] In the Supporting Information, we provided a detailed overview of relevant literature investigating the impact of SRT on the elimination of pharmaceuticals and other trace chemicals.

FACTORS INFLUENCING THE QUANTIFICATION OR THE PREDICTION OF ANTIBIOTIC REMOVAL IN WWTPS

Influent-Effluent Dynamics and Sampling. The release of down-the-drain chemicals (e.g., pharmaceuticals) into sewer systems and to WWTPs is inherently dynamic. Antibiotics are excreted and discharged in sewer pipelines as a result of discrete toilet flushes, resulting in short-term loading variations.[179−181] Attenuation of fluctuations is shown for substances exhibiting high sorption affinity onto solids in sewers.[182] At wider temporal scale, patterns in loading dynamics to WWTPs have

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been recognized for several pharmaceuticals and associated with (i) seasonal variations in pharmaceutical consumption and (ii) diurnal variations as a function of their administration patterns and half-life in human body.[42,183−186] Influent generator algorithms have been developed (using stochastic or deterministic approaches) to predict pharmaceutical loading in WWTP influents.[187]

Conventionally, the elimination of pharmaceuticals in full-scale WWTPs is calculated or predicted based on influent and effluent concentrations or loads. Capturing loading dynamics in WWTP influents and effluents is therefore crucial for an unbiased estimation of removal efficiencies, and is adequately achieved only by adopting the proper sampling strategy. Load variations, and consequently the selection of the sampling protocol, are influenced by several factors, namely, (i) the type of chemical, its usage and its pathway of discharge in the sewer network (determining the frequency and the shape of pulses through which the chemical is released in the sewer); (ii) the size and shape of the catchment (determining to what extent fluctuations are buffered as a result of in-sewer dispersion); (iii) the type of drainage system employed in the sewer network (determining variations of flow rates in WWTP influents).[180,188−191] According to the guidelines proposed by Ort et al.,[188] truly averaged concentrations of pharmaceuticals in influents and effluents of WWTPs serving middle-sized or large catchments can be measured by collecting daily flow-proportional composite samples. Additionally, the sampling error can be minimized by appropriately selecting the sampling frequency based on the expected number of pulses. The impact of WWTP residence time distribution on chemical concentrations in effluents may further require pooling of raw influent samples collected in consecutive days.[192] Sampling requirements to determine long-term (e.g., annual) influent loads of antibiotics have been further estimated.[186] Importantly, when assessing removal in specific sections of WWTPs, i.e. only during biological treatment, treatment steps located upstream (e.g., primary clarification) may contribute to reducing load fluctuations as compared to raw influent.[183]

For smaller catchments (e.g., hospitals), where the variation of concentrations is expected to be significantly higher due to reduced dispersion (shorter in-sewer residence time) or lower number of pulses expected (limited number of consumers),[193] high-frequency (<5 min) or continuous flow-proportional sampling may be required.[96,194]

Collection and analysis of composite and continuous wastewater samples involves the storage of wastewater in automatic samplers and (typically after filtration) in laboratories under refrigerated conditions (from −20° to 4 °C). The stability of trace chemicals, and more specifically antibiotics, during wastewater storage has been investigated elsewhere.[195−197] However, no conclusive evidence exists on the stability of SMX, CIP, and TCY and their human metabolites. Rather high instability of glucuronide conjugates (i.e., morphine-glucuronide) was shown in raw and filtered wastewater at 2 °C, and could be prevented by acidification.[195] Nevertheless, the use of acidification during wastewater collection (e.g., by means of preventive acid addition to sampling flasks) has been questioned due to the interference with pH-dependent sorption equilibria of ionizable chemicals, leading to changes in aqueous and sorbed concentrations.[195] Significant release of sorbed amounts to the aqueous phase due to pH decrease may for instance be expected for CIP.[124]

Laboratory-Scale Estimation of Process Parameters. Sampling campaigns in full-scale WWTPs are often complemented, if not replaced, with laboratory-scale experiments under controlled conditions. Targeted batch or continuous-flow experiments are used to derive fate parameters (namely, biotransformation rate constants, k_{Bio}, and solid−liquid partition coefficient, K_d), based on which full-scale elimination can be estimated. The reliability of estimated parameters is ensured only if experiments are carried out under environmentally representative conditions. We hereby identified and discussed a number of factors and associated pitfalls, some of which have been addressed elsewhere,[198] influencing observations made on pharmaceutical fate in laboratory-scale wastewater systems.

Underestimation of k_{Bio} by Measuring Parent Pharmaceutical Concentrations in Continuous-Flow and Batch Experiments. By monitoring concentrations of only parent pharmaceuticals in laboratory-scale experiments, retransformation processes leading to the formation of parent pharmaceuticals and thus masking their true elimination are in practice ignored. This may occur in continuous-flow experiments, when monitoring influent and effluent of biological reactors, or in batch experiments, when spiking only parent pharmaceuticals at concentrations similar to indigenous levels of retransformable chemicals.

Over- or Underestimation of Representative k_{Bio} in Batch Tests at Concentration Levels Significantly Higher than in the Environment. Pharmaceuticals, typically prevailing at trace levels in sewage and other environmental media, can be defined as nongrowth substrates. Although early studies[199,200] showed that laboratory-scale tests at concentrations greater than those in nature may not be representative of conditions typical of natural ecosystems, spiking of reference substances is still common practice.[201] Significant variations in measured removal efficiencies or estimated k_{Bio}, depending on the initial concentration level, were recently shown for several pharmaceuticals in wastewater, for example, ibuprofen,[175,202,203] diclofenac,[203] and clarithromycin.[175] A recent detailed study on trimethoprim[204] demonstrated that different spiking levels significantly influenced not only kinetics, but also pathways of biotransformation. Specifically, high concentrations of antibiotics may result in the inhibition of bacteria and/or enzymes responsible of their biotransformation, thus impacting transformation kinetics and pathways (as hypothesized for trimethoprim).[204] No significant variation of biotransformation kinetics (by biomass enriched in synthetic growth medium) was conversely shown at two different spiking levels of salicylic acid and trimethoprim under extant conditions.[142]

Over- or Underestimation of k_{Bio} from Experiments Run in the Excess of Growth Substrates. Typically present in wastewater in ng L^{-1} to μg L^{-1} concentration levels, pharmaceuticals are considered nongrowth substrates, not providing energy benefit to cells. Biological transformation is regarded as a cometabolic process, whereby primary substrates (COD, nutrients) support biomass growth and energy supply.[145,167,200,205−207] Under conditions of limiting growth substrate availability (e.g., at extended SRT), biotransformation may stem from mixed substrate utilization strategies[169−171] or fortuitous metabolism.[167,168] Furthermore, growth substrates can competitively inhibit or enhance the biotransformation of trace chemicals,[207] including pharmaceuticals.[32,33,198] Spiking of reference pharmaceuticals in batch or continuous-flow experiments involves the addition of carbon (e.g., in the form of methanol), in which

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spiked substances are dissolved. Biomass is thus provided with excess organic carbon in readily biodegradable form, thereby influencing observations of biological transformation by heterotrophic biomass[198] or ultimately causing a shift in microbial communities even in short-term batch experiments.[208]

Underestimation of k_{Bio} by Using Synthetic Growth Medium in Long-Term Experimental Studies. Biotransformation experiments are often conducted with biomass enriched by long-term feeding with synthetic wastewater. Such media often contain a limited range of growth substrates, as compared to real wastewater, thereby influencing the adaptation potential of biomass and minimizing the possible induction of gene expression, responsible for an effective heterotrophic biotransformation, by indigenous chemicals structurally similar to trace analytes.[201,205,209,210] For instance, enhanced estrogen biotransformation rates were found in the presence of recalcitrant organic substrate (mainly bacterial decay and lysis products), typically absent in synthetic media, as compared to reference readily biodegradable substrate.[210]

Overestimation of Sorption Coefficients (K_d) by Using Pharmaceutical Concentration in Solids, Possibly Propagating to k_{Bio} Estimates. Pharmaceutical concentrations present in solid phase (e.g., activated sludge) may include a sequestered fraction, not in equilibrium with aqueous concentrations and thus not potentially bioavailable following desorption. Desorption experiments[132,133] and modeling exercises[33] accordingly showed the significance of irreversibly sequestered fractions of pharmaceuticals in sewage sludge.

The influence of the factors described above on assessing pharmaceutical biotransformation is exemplified in Figure 1, where estimated k_{Bio} under aerobic and anoxic conditions for a range of pharmaceuticals are presented and compared. We distinguished between k_{Bio} values obtained (Figure 1a) in mixed culture experiments with activated sludge and real wastewater, without pharmaceutical spiking[32−34] and (Figure 1b) in enriched activated sludge fed with synthetic wastewater and spiking of reference substances.[143,211] In the latter case, more pronounced deviation between aerobic and anoxic k_{Bio} is noticed. Major discrepancies can be noticed, for example, for diclofenac (DCF).

MATERIALS AND METHODS

Description and Full-Scale Implementation of ASM-X. In the ASM-X version developed for antibiotics,[32] three states are used to describe the concentrations (ng L^{-1}) of different fractions: (i) the dissolved parent fraction (C_{LI}), (ii) the sorbed parent fraction (C_{SL}), and (iii) the retransformable fraction (C_{CJ}). The variable C_{CJ} identifies chemicals undergoing retransformation to C_{LI} via the processes described above. Fate processes include the biotransformation of C_{LI}, retransformation of C_{CJ} back to C_{LI} and sorption (desorption) onto (from) sludge. The parameters used in process rate equations include the biotransformation rate constant, k_{Bio} (L $gTSS^{-1}$ d^{-1}), the retransformation rate constant, k_{Dec} (L $gTSS^{-1}$ d^{-1}), the solid−liquid partition coefficient, K_d (L $gTSS^{-1}$), and the desorption rate, k_{Des} (d^{-1}). A detailed description of the ASM-X structure, including state variables and parameters, is provided in the Supporting Information (Table S2).

A parameter is defined to characterize the parent-to-retransformable ratio in influent sewage, that is (eq 1),

$$n_{LI,CJ} = C_{LI,in}/C_{CJ,in} \quad (1)$$

where $C_{LI,in}$ and $C_{CJ,in}$ denote the influent concentration of parent and retransformable fractions, respectively.

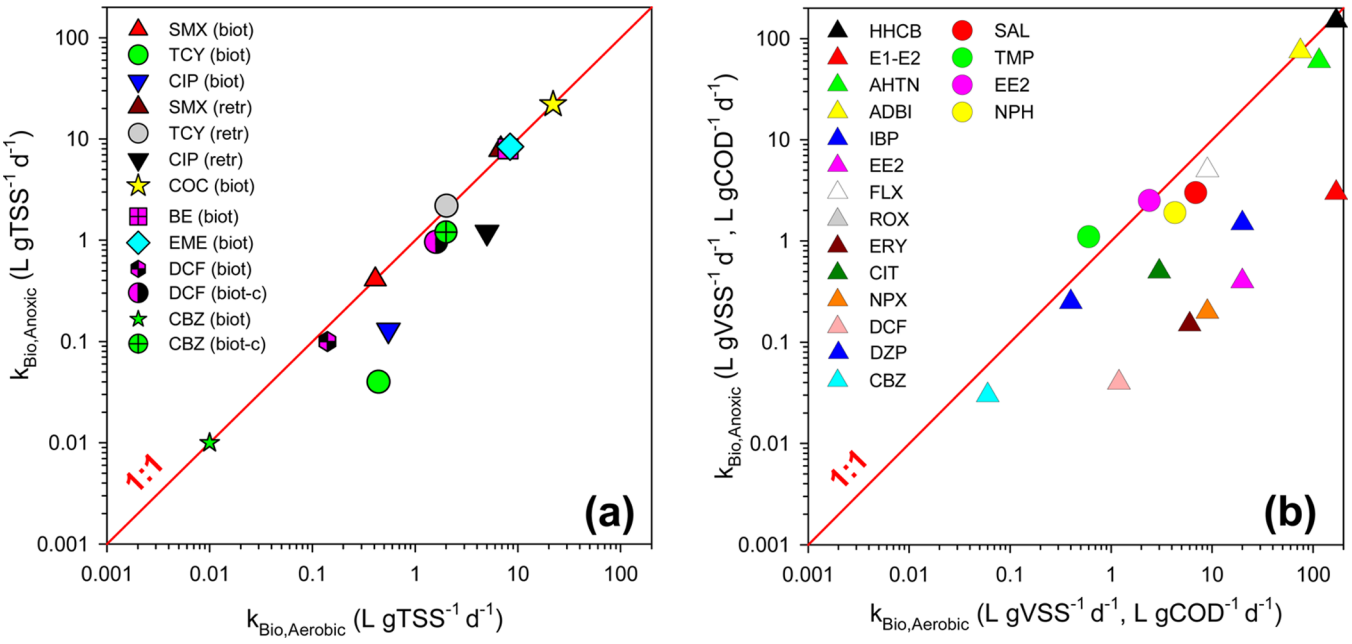

Figure 1. Comparative assessment of transformation rate constants estimated under aerobic and anoxic denitrifying conditions by (a) Plósz et al.[32−34] and (b) Suárez et al.[143] (triangles) and Su et al.[211] (circles). Reported values refer to rate constants for biotransformation (biot, k_{Bio}), cometabolic biotransformation in the presence of growth substrate (biot-c, q_{Bio})[33] and retransformation (retr, k_{Dec}). Substances assessed include: sulfamethoxazole (SMX), tetracycline (TCY), ciprofloxacin (CIP), cocaine (COC), benzoyl-ecgonine (BE), ecgonine-methyl-ester (EME), diclofenac (DCF), carbamazepine (CBZ), galaxolide (HHCB), natural estrogens E1 and E2, tonalide (AHTN), celestolide (ADBI), ibuprofen (IBP), 17α-ethinyl estradiol (EE2), fluoxetine (FLX), roxithromycin (ROX), erythromycin (ERY), citalopram (CIT), naproxen (NPX), diazepam (DZP), salicylic acid (SAL), trimethoprim (TMP), and nonylphenol (NPH).

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The full-scale ASM-X implementation in WEST 2012 (DHI, Denmark), with parameters (k_{Bio}, k_{Dec}, K_d, $n_{LI,CJ}$) estimated from batch experimental results and evaluated with measurements performed in a 3-day sampling campaign in Bekkelaget WWTP (Oslo, Norway), was used to estimate the elimination of the selected antibiotics. Further details on biological treatment in Bekkelaget WWTP are presented in the Supporting Information and elsewhere.[32,33,183] For a meaningful comparison with literature data, scenario simulations were performed using increased or decreased influent loads of pharmaceuticals, as compared to full-scale measurements.[32]

Generalization: Concepts. *Removal Efficiency Calculation.* The full-scale removal efficiency of SMX, CIP, and TCY was calculated based on preclarified and secondary effluent concentrations of these substances. We distinguished between removal efficiency [−] based only on the dissolved parent fraction (parent-based removal efficiency η_{LI}, eq 2)

$$\eta_{LI} = (M_{LI,in} - M_{LI,eff})/M_{LI,in} \quad (2)$$

and on both parent and retransformable fractions (total removal efficiency η_{TOT}, eq 3)

$$\eta_{TOT} = (M_{LI,in} + M_{CJ,in} - M_{LI,eff} - M_{CJ,eff}) / (M_{LI,in} + M_{CJ,in}) \quad (3)$$

where M denotes chemical mass load ($\mu g\ h^{-1}$ or $\mu g\ d^{-1}$), the subscripts "LI" and "CJ" identify parent and retransformable fractions, respectively, and the subscripts "in" and "eff" identify secondary influent and effluent, respectively. Estimated η_{LI} and η_{TOT} were plotted as a function of the normalized influent load of pharmaceuticals (mg h^{-1} 1000 PE^{-1}), which was calculated from the influent flow rate (Q_{in}) and design capacity in terms of PE of the WWTP. We note that the design PE does not correspond to the population served by each WWTP, and is used to allow for a comparison between measured data from differently sized WWTPs and predicted removal efficiencies.

Review of Published Removal Data. A literature review was performed to collect removal efficiency data for SMX, CIP and TCY. International data on pilot- and full-scale removal of pharmaceuticals were included in this study only if the corresponding literature source responded to a number of criteria: (i) the wastewater sampling protocol used was explicitly reported and data presented were obtained by applying sound sampling techniques (see Influent–Effluent Dynamics and Sampling section), based on information provided in the publication; (ii) removal efficiencies during secondary treatment could be calculated, where not explicitly reported, from published data (e.g., concentrations in secondary influent and effluent); (iii) additional information on the WWTP assessed was sufficiently detailed, including average influent flow rate of the WWTP or influent flow rate in the sampling period, HRT, SRT, and the design capacity in terms of PE. To our knowledge, a number of literature studies[46,55,56,104,105,121,159] complied with these criteria. These studies investigated pharmaceutical elimination in conventional activated sludge (CAS), fixed bed biofilm reactors (FBR) and membrane bioreactors (MBR). In addition, measurements by Yang et al.[212] in secondary WWTP influent were used to discuss variability of the parameter $n_{LI,CJ}$ (eq 1) in full-scale WWTPs. Further details on full-scale WWTPs and catchments investigated in the mentioned literature studies are presented in the Supporting Information (Tables S5–S6).

Zero-Catchment Scenario. Literature studies on in situ treatment of hospital effluent were selected to characterize a "zero-catchment" scenario, describing the removal of pharmaceuticals in WWTPs with negligible upstream sewer transport. Literature data[96,213,214] were used to assess antibiotic removal in WWTPs treating hospital wastewater (MBR, hybrid biofilm-activated sludge, HYBAS and moving bed biofilm reactor, MBBR respectively) and compared with ASM-X predictions. In this way, we aimed at addressing the potential impact of retransformation processes, not occurring in upstream sewer but only via biological treatment, on the observed elimination. As hospital WWTP capacity is usually expressed in terms of hospital beds, predictions and measured data were compared assuming that 1 bed was equivalent to 3.4 PE[96] based on the wastewater production per bed in the study and the wastewater production per PE (200 L d^{-1} PE^{-1}).[215]

Presentation of Results. ASM-X generalization was performed by plotting estimated η_{LI} and η_{TOT} against the normalized influent load of pharmaceuticals. An optimal way to present these results was found in operating plots that were constructed using 6-h average influent loads and removal efficiency (Figure S5).[33] In this way, we accounted for two crucial factors, influencing the calculation of removal efficiencies: (i) dynamics in the influent loads of pharmaceuticals, observed during the sampling period; and (ii) residence time and consequent dispersion effect in the simulated WWTP.[192] Removal efficiency predictions were presented under a range of loading conditions, and could be used for comparison with literature removal data, usually referred to 24-h up to 3-d composite samples. In Figure S5, predicted removal efficiency curves are defined using the mean of predictions (dashed black line) and an uncertainty interval (light or dark gray) based on standard deviation of 6-h removal efficiency predictions. Examples of the interpretation of operating plots are directly provided in the Results and Discussion section and further details are given in the Supporting Information.

Dynamic Risk Assessment. A preliminary assessment of the environmental risk, associated with the release of residual pharmaceuticals to surface water, was performed. Based on ASM-X predictions,[32] predicted environmental concentrations (PECs) were estimated from Bekkelaget secondary effluent concentration (eqs 4 and 5)

$$PEC_{LI} = C_{LI,eff}/DF \quad (4)$$

$$PEC_{TOT} = (C_{LI,eff} + C_{CJ,eff})/DF \quad (5)$$

where $C_{LI,eff}$ and $C_{CJ,eff}$ (ng L^{-1}) denote the measured secondary effluent concentrations of parent and retransformable fraction, respectively.

We distinguished between PECs accounting for only effluent C_{LI} (PEC_{LI}) and for both effluent C_{LI} and C_{CJ} (PEC_{TOT}). A default dilution factor (DF) of 10 was assumed for the estimation.[216] Quasi-MECs (concentrations in freshwater estimated from measurements in WWTP effluent[1]) were also estimated from measured C_{LI} in secondary effluent.[32] PECs and quasi-MECs were finally compared to predicted no-effect concentrations (PNECs) reported in literature, describing threshold concentrations for risk in receiving water bodies. Risk was associated with a pharmaceutical in Bekkelaget effluent when the ratio between PEC (or quasi-MECs) and PNEC (also known as risk quotient, RQ) was greater than 1. Additionally, the dynamics in effluent concentrations (and therefore, in PECs

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and quasi-MECs) allowed for the assessment of trends and extents of the risk during the sampling period.

RESULTS AND DISCUSSION

Sulfamethoxazole (SMX). *Removal in Full-Scale WWTPs.* The elimination of parent SMX has been previously investigated in conventional and advanced biological full-scale WWTPs. Biotransformation has been recognized as the main elimination mechanism of SMX,[32,55,56,123,159] with minor contribution from sorption onto sludge. Among all conjugated metabolites of SMX, detailed evidence on the full-scale removal of only N_4-acetyl-SMX is available.[55,56] Sorption of N_4-acetyl-SMX was found to be negligible because of its high solubility and polar nature (typical of conjugated metabolites). Considering the absence of measurements on other conjugated metabolites of SMX, namely SMX-N_1-Glu, the retransformable fraction C_{CJ} of SMX in literature studies was assumed to be entirely represented by N_4-acetyl-SMX. We accordingly compared predicted η_{TOT} with the combined removal efficiency of SMX and N_4-acetyl-SMX (also referred to as η_{TOT} in Figure 2).

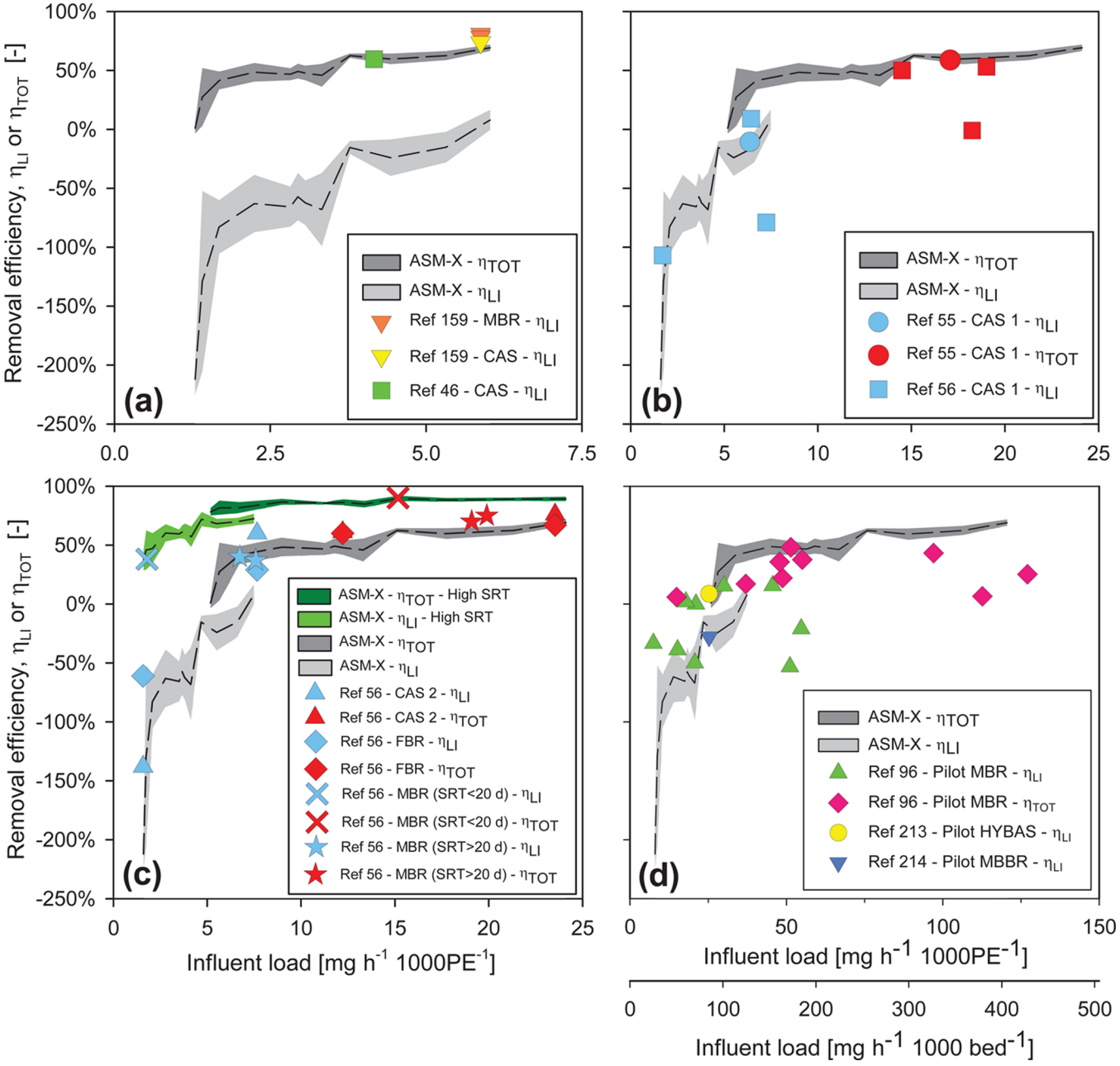

Figure 2. Operating plots showing parent-based (η_{LI}) and total removal efficiencies (η_{TOT}) for SMX predicted by ASM-X and measured in full- and pilot-scale WWTPs. Removal efficiencies are plotted as a function of the influent load of the antibiotic normalized by the WWTP capacity. The comparison between measured and predicted removal efficiencies is presented according to the following subdivision: (a) literature studies presenting only parent-based removal efficiency; (b–c) literature studies presenting parent-based and total removal efficiencies in systems at SRT (b) lower than 16 d and (c) higher than 16 d; (d) literature studies assessing hospital WWTPs in the "zero-catchment scenario". SRT values for WWTPs considered in this figure are reported in Table S5. Measured removal efficiencies denoted as η_{TOT} include the elimination of parent SMX and the conjugated metabolite N_4-acetyl-SMX. Scenario simulations to derive predicted η_{LI} and η_{TOT} in panels a, b–c, and d were run considering a 1.25-, 5-, and 25-fold increased influent load, respectively. Scenario simulations to derive predicted η_{LI} and η_{TOT} at extended SRT in panel c were run assuming k_{Bio} = 3 L $gTSS^{-1}$ d^{-1}.

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In Figure 2, ASM-X removal efficiency predictions (shaded curves) are compared with measured removal efficiency (data points) for SMX (and N_4-acetyl-SMX, where possible). Predicted removal efficiency (η_{LI} and η_{TOT}) curves were obtained simulating a 1.25-fold (Figure 2a) and 5-fold (Figure 2b−c) increased influent loading of SMX as compared to Bekkelaget WWTP. For a meaningful comparison, we distinguished between literature studies assessing the elimination of parent SMX only (Figure 2a) and literature studies assessing the elimination of both SMX and N_4-acetyl-SMX in WWTPs operating at SRT lower than and 16 d (Figure 2b) and higher than 16 d (Figure 2c). A threshold of 16 d represents the SRT, at which Bekkelaget WWTP was operated during the sampling campaign used for ASM-X evaluation.[32,33]

Overall, a large variability in measured removal efficiency for SMX (from −150% to 80%) is evident from published data. To explain the observed variability, studies assessing only the elimination of parent SMX[46,159] are first considered (Figure 2a). Measurements are well approximated by η_{TOT} predictions, indicating limited impact of retransformation during secondary treatment as a result of low influent C_{CJ} concentrations. This hypothesis is supported by (a) the significant formation (+45%) of SMX during primary settling;[46] (b) the input load contribution from pharmaceutical manufacturing to the WWTP investigated;[159] (c) reduced concentrations of conjugates of other sulfonamides (acetyl-sulfamethazine) in the primary effluent in the same WWTP;[217] (d) the size of the WWTPs investigated (Table S5), serving medium-to-large-sized catchments with likely retransformation in upstream sewers. The significance of (d) is discussed later in the manuscript.

ASM-X predictions of η_{LI} and η_{TOT} are in close agreement with removal efficiencies for SMX and SMX + N_4-acetyl-SMX, respectively, reported for Kloten−Opfikon WWTP[55,56](CAS 1, Switzerland; SRT < 16 d; Figure 2b). Low or negative η_{LI} (from −107% to 9%) occurred as a result of the almost complete retransformation, through deconjugation, of N_4-acetyl-SMX back to parent SMX. The underestimation error between η_{LI} and η_{TOT} in Kloten-Opfikon (from −160% to 44%) was in the error range predicted by ASM-X (from −200% to 60%). The good agreement between measured (including N_4-acetyl-SMX as the only conjugate) and predicted η_{TOT} indicates that the retransformable fraction in WWTPs may be almost completely represented by the acetylated conjugate. Accordingly, estimated k_{Dec} values[32] well approximate biotransformation rate constants for N_4-acetyl-SMX in activated sludge (Table S4). The other conjugated metabolite SMX-N_1-Glu may undergo (i) extensive and fast retransformation in upstream sewers, with negligible presence in WWTP influent or (ii) limited retransformation (and elimination) during biological wastewater treatment.

Figure 2c presents η_{LI} and η_{TOT} predictions and measurements in WWTPs operating at SRT higher than or equal to 16 d (16−80 d). Although SRT is not determined for attached growth systems, removal efficiency data measured in FBR[56] were also considered. Significant variability is shown for measured η_{LI} (from −138% to 60%) due to observations in CAS2 WWTP. Minimum η_{LI} for CAS 2 (−138%) and FBR (−61%) occurred when comparably higher loads of N_4-acetyl-SMX ($n_{LI,CJ}$ = 0.15) occurred in secondary influent. Again, the reported data highlight the significant underestimation error arising by characterizing SMX removal only based on parent SMX concentration. Importantly, measured η_{LI} and η_{TOT} included in Figure 2c showed an overall increase when compared to what presented in Figure 2b. Measured η_{TOT} (60%−76%) in CAS 2 and FBR were generally higher than in CAS 1 (from −1% to 53%). In addition, measured η_{LI} (37%−40%) and η_{TOT} (70%−90%) in MBR (SRT = 16−80 d)[56] were consistently higher than for CAS1 (Figure 2b) under similar loading conditions (the two systems were operated in parallel). As a consequence, most of the measured η_{LI} and η_{TOT} were higher than respective prediction curves, indicating that retransformation per se may not explain the variability in observed removal efficiencies. We hypothesized that a kinetic improvement of SMX biotransformation occurred in WWTPs operated at SRT ≥ 16 d. A scenario simulation with ASM-X was performed assuming increased biotransformation rate constant for parent SMX (k_{Bio} = 3 L $gTSS^{-1}$ d^{-1}; Figure 2c, "High SRT" curves) to estimate and reproduce enhanced η_{LI} and η_{TOT}. The obtained predictions were selected to describe the highest measured removal efficiencies (MBR with SRT < 20 d).[56] The estimated k_{Bio} is a comparably high value and the simulated increase possibly lumps other effects. We note that the increased biomass concentration at which MBRs are typically operated (2−3-fold higher TSS than in conventional WWTPs) cannot alone explain the observed enhancement in SMX elimination. Accordingly, no further increase of η_{LI} or η_{TOT} as a function of SRT was observed (at SRT > 16 d) in the MBR.[56]

Overall, the model-based assessment of SMX elimination in WWTPs suggests that the variability of observed removal efficiency can be associated with the influence of conjugated metabolites (namely, N_4-acetyl-SMX) in secondary influents and of the SRT set for WWTP operation. Such conclusions were drawn only when removal of SMX and N_4-acetyl-SMX were simultaneously assessed, and could be used to explain observations in other WWTPs.[46,159]

Zero-Catchment Scenario. Figure 2d presents η_{LI} and η_{TOT} curves predicted with ASM-X (25-fold increased influent loading of SMX compared to Bekkelaget WWTP), and published removal efficiency measurements.[96,213,214] In these studies, a pilot MBR (SRT = 30−50 d, TSS = 2 g L^{-1}) and two biofilm systems (HYBAS and MBBR), respectively, were operated for decentralized treatment of hospital effluent. In one study[96] aqueous concentrations of both SMX and N_4-acetyl-SMX were measured, allowing for the calculation of η_{LI} and η_{TOT}. The deviation between η_{LI} and η_{TOT} was also in this case significant (from 22% to 72%), in the error range predicted by ASM-X. Both η_{LI} (from −53% to 26%) and η_{TOT} (from 6% to 48%) were lower than in the previously assessed MBR but comparable to efficiencies achieved in CAS and FBR systems. Measured η_{LI} data from the three studies were generally underpredicted by η_{LI} curve, whereas measured η_{TOT} data points were located on or below the corresponding η_{TOT} curve. Considering the significant elimination of N_4-acetyl-SMX (81% ± 4%), the absence of catchment between the hospital effluent and the pilot MBR could only to some extent explain the observations. Influent $n_{LI,CJ}$ to the MBR (≥1 g_{LI} g_{CJ}^{-1}) was on average higher than in urban catchments[32,55,56] and excreted ratios expected from human pharmacokinetics (Figure S1). Comparably lower concentrations of N_4-acetyl-SMX in secondary influent may therefore explain the generally higher η_{LI} measurements as compared to ASM-X predictions. No evidence of deconjugation in the primary clarifier, located upstream to the MBR, was reported,[96] indicating that specific $n_{LI,CJ}$ ratios were intrinsic to the hospital effluent (excluding that retransformation occurred during sample storage and handling). We provided a possible interpretation of observations by comparing $n_{LI,CJ}$

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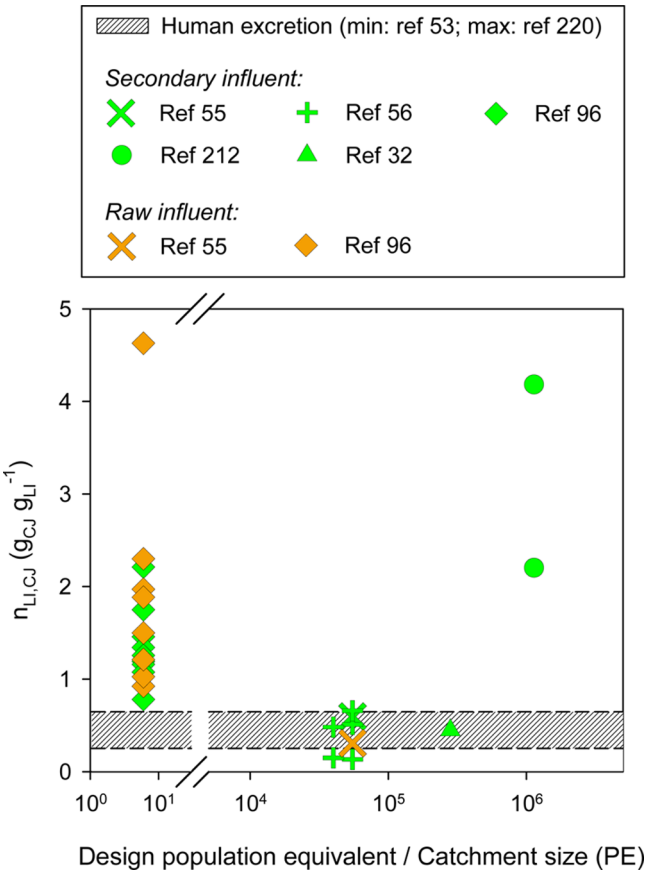

Figure 3. Influent parent-to-retransformable ratios ($n_{LI,CJ}$) for SMX in pilot- and full-scale WWTPs as a function of catchment size. The catchment size is here used to assess the extent of retransformation of conjugated SMX back to parent SMX in sewers upstream of full-scale WWTPs and its influence on raw and secondary influent composition in terms of parent and conjugated SMX (expressed by $n_{LI,CJ}$). Design capacity in population equivalents (PE) of studied WWTPs is here considered as an indicator of catchment size.

data from municipal and hospital WWTPs (see Impact of Retransformation/In-Sewer Retransformation section, Figure 3). As shown by the comparison between measured and predicted η_{TOT}, the extended SRT in MBR, HYBAS, and MBBR could not explain the enhancement of parent SMX elimination.

Impact of Retransformation/In-Sewer Retransformation. By comparing measured removal efficiencies to η_{LI} and η_{TOT} prediction curves (Figure 2), it was possible to quantify the impact of retransformation from SMX conjugates back to parent SMX in pilot- and full-scale biological WWTPs. Variability in measured SMX removal efficiencies was explained by different parent-to-retransformable ratios ($n_{LI,CJ}$) in secondary influent due to transformation processes in sewer networks upstream of WWTPs. To date, in-sewer biological transformation processes (or "reactive sewer" concept) have been systematically evaluated only for limited chemical groups, for example, estrogens and their excreted conjugates.[40,69,218] On the contrary, in-sewer processes have been considered not relevant for antibiotics,[186] whereas recent evidence[219] showed significant formation of SMX (+66%) during transport in pressurized sewer pipelines.

The extent of in-sewer retransformation of SMX conjugates likely depends on: (i) the mean hydraulic retention time of raw sewage in sewer pipelines; and (ii) redox conditions prevailing in sewer systems. Very little evidence is available as to point (ii) because only anaerobic conditions have been assessed[219] and no information on transformation kinetics in raw sewage is available. Mean residence times in upstream sewers are also not typically reported in full-scale fate studies, making an evaluation of their impact challenging.

Therefore, we evaluated the variability of $n_{LI,CJ}$ for SMX in worldwide full-scale WWTP influents as a function of the WWTP design capacity (in PE) (Figure 3). It was again assumed that the retransformable fraction of SMX (C_{CJ}) would coincide with N_4-acetyl-SMX concentration. The design capacity is used here as an approximation of the catchment size and is considered as a surrogate indicator of the mean residence time in sewer systems. Our evaluation did not aim at establishing mathematical correlations, and is based on a number of assumptions/simplifications: (a) redox conditions do not influence kinetics of SMX retransformation, as preliminarily confirmed by k_{Dec} values from various literature sources (Table S4); (b) SMX consumption and excretion is homogeneously distributed within a catchment; (c) differences in residence time in gravity and pressurized sewers are not considered; (d) differences in population density within studied catchments are insignificant. A preliminary assessment showed that the design capacity can give an indication of in-sewer mean residence time (Table S7). Measured $n_{LI,CJ}$ in both raw and preclarified sewage were included (Figure 3), considering evidence of deconjugation in primary settlers.[46,55,56] Measured $n_{LI,CJ}$ were compared with the range of expected ratios at the excretion point.[53,220]

We first considered $n_{LI,CJ}$ in the influent of full-scale municipal WWTPs (design capacity > 10 000 PE). Measurements by Göbel et al.[55,56] approximated theoretical excreted ratios, indicating limited retransformation in a small-sized catchment (Table S7). A similar ratio was estimated for a larger catchment (WWTP capacity = 281 000 PE) using a model-based assessment.[32] Significantly higher $n_{LI,CJ}$ (>2 g_{LI} g_{CJ}^{-1}) was measured in the secondary influent of large WWTP[212] (capacity = 1 000 000 PE), indicating that extensive retransformation may have occurred upstream to the sampling point. These preliminary findings suggest that influent composition in terms of parent and retransformable fractions, and thus the extent of retransformation upstream to secondary treatment, may depend on the catchment size. This could further explain the observation of higher η_{LI} measurements (comparable to predicted η_{TOT}) in WWTPs with 277 000 PE[159] and 740 000 PE[46] (Figure 2a).

Measured $n_{LI,CJ}$ in raw and preclarified hospital effluent[96] were generally found higher than theoretical ratios at the excretion point, thus explaining the underestimation of η_{LI} measurements[96,213,214] by ASM-X predictions (Figure 2d). This evidence was not expected based on our hypothesis that negligible in-sewer transport would correspond to limited retransformation upstream to secondary treatment. We explored alternative interpretations, focusing on SMX consumption and excretion in hospitals and households. The fraction of SMX consumed or excreted in hospitals, compared to the overall consumption/excretion in a specific catchment, exhibits significant geographical (Norway, < 1%;[221] Australia and Italy, < 10%;[194,222,223] Germany, 12%−18%;[224] Switzerland, 16%−52%;[225] Denmark, ~100%, medstat.dk for year 2013) and temporal variations.[225] Even when administration occurs in hospitals, not negligible fractions of pharmaceuticals may still be excreted in households because of specific treatments or reduced hospitalization periods.[194,226]

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Thus, only a limited fraction of pharmaceuticals administered in hospital effectively reaches hospital effluents. Pharmacokinetic studies have indicated slower elimination of N_4-acetyl-SMX (i.e., longer half-life) from human body than of parent SMX in healthy[54,227] and renally impaired subjects.[228] Accordingly, relative excreted amounts of SMX and N_4-acetyl-SMX in urine vary from the initial hours (e.g., in hospitals) to 48−96 h (e.g., in households) after administration and, more importantly, higher $n_{LI,CJ}$ can be expected in hospital sewage. Notably, theoretical $n_{LI,CJ}$ at the excretion point, derived from data in Table S1, represent average values over 60−96 h after SMX administration.

Overall, our observations point at the following conclusions: (i) the impact of SMX formation during wastewater treatment is more pronounced in small urban catchments/WWTPs, where limited retransformation in upstream catchments is expected, and (ii) temporal trends for the excretion of SMX and its conjugated metabolites may have a significant impact on loads entering WWTPs that treat hospital sewage.

Impact of SRT and MBR Operation. By comparing predicted and measured[56] η_{LI} and η_{TOT}, it was possible to identify enhanced removal efficiencies in municipal CAS, FBR, and particularly, MBR WWTPs (SRT = 16−80 d). For the MBR system, a comparison with removal efficiencies in a CAS WWTP operated in parallel confirmed that variability in observations was not due to different $n_{LI,CJ}$ ratios in influent. Considering the almost complete removal of N_4-acetyl-SMX (>80%) in all WWTPs investigated, a kinetic improvement of SMX biotransformation at SRT > 16 d likely caused the enhanced SMX elimination. Through scenario simulations, we suggested that improved biotransformation kinetics resulted from a step increase of k_{Bio}, in agreement with previous findings for diclofenac.[33]

In previous studies at SRT > 100 d,[175,176] estimated k_{Bio} values for SMX for laboratory- and pilot-scale MBRs were found comparable to CAS (see Table S4 for comparison). This may though be considered as limited evidence of the influence of extended SRT on biotransformation kinetics of SMX. Improved SMX elimination may be alternatively attributed to higher TSS concentrations, at which MBRs are typically operated.[161] Notably, k_{Bio} values estimated to date (Table S4) were normalized by the total biomass content (as TSS or VSS), not accounting for the reduction of the active biomass fraction at increasing SRT.[57] Thus, it should not be excluded that the biotransformation capacity of active biomass may be enhanced at prolonged SRT (as a result of increased biodiversity or improved metabolic capabilities)—a hypothesis that could be demonstrated only by assessing k_{Bio} normalized by active biomass.

Overall, currently available evidence is still limited to provide a valuable explanation of observed SMX elimination and thus to support the hypothesis proposed in this study.

Ciprofloxacin. *Removal in Full-Scale WWTPs.* The fate of CIP was investigated in CAS WWTPs,[104,105,121] where the elimination almost entirely occurred via sorption onto activated sludge. In Figure S6a, ASM-X prediction curves for η_{LI} and η_{TOT} were compared to measured removal efficiencies only for parent CIP.[104,105,121] Scenario simulations with ASM-X were run assuming reduced influent loading (1/30th of original loading[32]). Sorption equilibria in activated sludge were simulated using the Freundlich isotherm equation (eq 6)

$$C_{SL} = K_f C_{LI}^{\ n} / X_{SS} \quad (6)$$

where X_{SS} (gTSS L^{-1}) denotes the TSS concentration of activated sludge and experimental values for the Freundlich coefficients K_f (10.6−10.7 μg^{1-n} L^n g^{-1}) and n (0.62−0.73) under anoxic and aerobic conditions were used.[124] Implementation of eq 6 in ASM-X rate equations is presented in Table S2. Measured CIP removal efficiencies were in good agreement with ASM-X predictions for η_{TOT}, indicating limited retransformation and/or negligible concentrations of the retransformable fraction (C_{CJ}) in the WWTPs investigated.

Experimental and modeling studies[13,59,104,105,121,229,230] showed the presence of relevant amounts of CIP sorbed onto influent and effluent solids. To further assess ASM-X predictions when accounting for sorbed fractions, further scenario simulations were run, assuming (i) negligible retransformable fraction in the influent ($C_{CJ,in}$ = 0); (ii) sorbed CIP concentrations in secondary influent solids based on measured influent TSS and CIP concentrations[32] and K_d = 2 L $gTSS^{-1}$ for primary sludge.[105,121] On the basis of ASM-X predictions, we calculated a removal efficiency ($\eta_{TOT,X}$) that accounts for influent and effluent loads of CIP sorbed onto solids (eq 7):

$$\eta_{TOT,X} = (M_{LI,in} + M_{SL,in} - M_{LI,eff} - M_{SL,eff}) / (M_{LI,in} + M_{SL,in}) \quad (7)$$

where $M_{SL,in}$ and $M_{SL,eff}$ denote the mass loads of sorbed CIP onto secondary influent and secondary effluent solids, respectively, and $M_{LI,in}$ and $M_{LI,eff}$ the mass loads of parent CIP in secondary influent and effluent, respectively. Similarly, reported CIP concentrations in sorbed phase were used to calculate measured $\eta_{TOT,X}$ from full-scale investigations.[104,105,121] Predicted η_{LI} and $\eta_{TOT,X}$ were in good agreement with measured CIP removal efficiencies (Figure S6b−c). The deviation between η_{LI} and $\eta_{TOT,X}$ is shown to be rather small for ASM-X predictions (0%−2%) and some of the full-scale measurements (4%−5%[105,121]), whereas significant deviation (35%) was also reported.[104] Although small compared to SMX, the variability of measured η_{LI} and $\eta_{TOT,X}$ for CIP may be attributed to uncertainties intrinsic to analytical methods, in particular to the extraction and quantification in solid matrices.[231]

Zero-Catchment Scenario. CIP elimination was also investigated in MBR treating hospital sewage.[96] Measured removal efficiencies, only referring to CIP in the aqueous phase, were compared to ASM-X predictions for η_{LI} and η_{TOT} (Figure S7a). Scenario simulations were performed assuming 4-fold increased influent loading using a Freundlich-based sorption model (see above). Measured η_{LI} (29%−72%) were close to η_{TOT} predictions, being significantly higher than predicted η_{LI} (lower than −150%). In analogy with findings for full-scale WWTPs (Figure S6a), our results suggest insignificant retransformation or alternatively the presence of negligible retransformable CIP fraction. CIP removal prediction was consequently assessed via new scenario simulations with ASM-X, considering the retransformable fraction in secondary influent negligible ($C_{CJ,in}$ = 0) and the presence of sorbed CIP concentration in secondary influent solids (K_d = 2 L $gTSS^{-1}$). New η_{LI} (and $\eta_{TOT,X}$, accounting for sorbed fractions in influent and effluent) predictions were obtained, in agreement with measured η_{LI} (Figure S7b). We note that removal efficiencies measured in the hospital MBR (Figure S7) were on average lower than in full-scale WWTPs (Figure S6). CIP concentration in sorbed phase was not measured, preventing a complete mass balance. Nevertheless, reduced removal efficiency may be attributed to less efficient sorption (resulting from competition

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for or progressive saturation of sorption sites by pharmaceuticals at $\mu g\ L^{-1}$ levels) or to the presence of conjugates (sulfo-CIP) in influent. CIP elimination was also investigated in a full-scale WWTP (primary clarification + MBR) treating hospital effluent.[232,233] The investigation highlighted (a) the relevance of sorption as removal mechanism and (b) an ~10% deviation between theoretical excreted CIP loads and influent loads to WWTP, possibly corresponding to the amounts of CIP excreted in faeces and/or in conjugated form (sulfo-CIP) (Figure S4 and Table S1).

Tetracycline. *Removal in Full-Scale WWTPs.* Elimination of TCY during biological wastewater treatment occurs via sorption onto solid matrices (e.g., sludge), with limited contribution from biotransformation.[32,122,123] As previously described for CIP, TCY exhibits significant affinity to solids despite its low hydrophobicity (log D = −1.25 at pH 7; ACD/Laboratories LogD). To our knowledge, no study documenting TCY elimination during secondary wastewater treatment satisfied the criteria set in this study for preliminary data screening, e.g. the use of adequate sampling protocols. The relevance of residual TCY in WWTP effluents for environmental risk assessment was thus discussed based only on simulation results (see following paragraph). Evidence from batch studies[32,122,123] suggests significant TCY removal from the aqueous phase, as confirmed by full-scale measurements[32] (η_{LI} = 77% ± 8%). Similarly to CIP, variability in removal efficiency determinations for TCY may be expected due to uncertainties associated with analytical methods, including, for example, the quantification of TCY in solids and of matrix effects in influent and effluent wastewater.[231,234]

Implications for Environmental Risk Assessment. Environmental risk assessment (ERA) methodologies are used to characterize the potential risk for ecological receptors from the exposure to toxic chemicals released in the environment. ERAs rely on the prediction of environmental concentrations (PECs) at regional or local catchment level using a range of modeling tools[235] and on their comparison to PNECs derived from ecotoxicological experiments. Guidelines for ERAs of pharmaceuticals have been proposed by the European Medicines Agency (EMA) with integration from the REACH (Registration, Evaluation, Authorisation, and Restriction of Chemicals) framework regulation.[236] Emissions from WWTPs to receiving water bodies are recognized as one of the major sources of ecological exposure to pharmaceuticals.

A number of recommendations for the improvement of these guidelines have been recently proposed.[237−241] Importantly, ERAs are typically conducted by simulating nondynamic release of pharmaceuticals from WWTPs, thus estimating steady-state PECs. This appears to be a rather simplified approach when considering (a) significant intraday variations in effluent loads and concentrations of antibiotics;[183,242] (ii) differences in antibiotic consumption rates over the year.[225] In addition, the release of retransformable pharmaceutical fractions may represent an additional source of risk, due to the potential formation of parent pharmaceuticals in freshwater. Inclusion of retransformable metabolites in ERA had been previously recommended for estrogens.[50,243]

Considering these shortcomings, we present here an example of ERA where dynamics in PECs and the contribution of the retransformable fraction are accounted for when assessing environmental risk in surface water. Specifically, we present (Figure 4) the results of a preliminary ERA on the release of TCY to receiving surface water from Bekkelaget WWTP

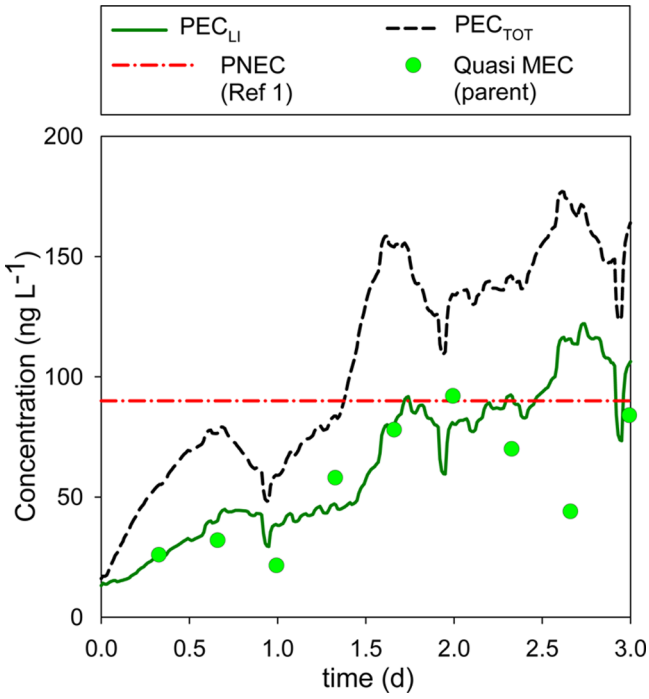

Figure 4. Dynamic risk assessment of TCY in surface water considering only parent (C_{LI}) and parent + retransformable fractions (C_{TOT} = C_{LI} + C_{CJ}) released from WWTP. PEC_{LI} (green solid line) and PEC_{TOT} (black dashed line) were calculated from ASM-X predictions for effluent TCY (dilution factor = 10[216]). PNEC of parent TCY was obtained from acute toxicity data.[1,221] Quasi-MECs (circles) were estimated from measured parent TCY concentrations in Bekkelaget WWTP effluent.[32] The evaluated time interval (3 d) refers to the full-scale monitoring of Bekkelaget WWTP, used also for ASM-X validation.[32]

effluent. Quasi-MECs, PEC_{LI} and PEC_{TOT} were calculated based on three-day monitoring and modeling predictions[32] and compared to PNEC (acute toxicity data[1]). Two major evidence sets are highlighted: (i) the significant variations of quasi-MEC and PEC_{LI}, temporarily exceeding the PNEC threshold in the three-day time interval; (ii) the potential contribution of the retransformable fraction to PEC_{TOT} and thus to the overall risk associated with TCY. We note that our evaluation as to point (ii) should be considered as speculative, since TCY retransformation could not be addressed in this generalization study by comparing ASM-X predictions to international full-scale measurements. Furthermore, simulation results should be regarded as preliminary since a fixed dilution factor was assumed and more complex hydrodynamic phenomena (downstream transport, dispersion) were not considered. Nevertheless, considering dynamics in WWTP effluents and monitoring through multiple days may be relevant for improvement of risk assessment protocols, especially for acute toxicity effects and in freshwater streams having significant contribution from WWTP effluents.[237] In addition, the presence of potentially retransformable fractions of pharmaceuticals can amplify the ecological risk, and this hypothesis requires further investigation.

OUTLOOK AND IMPLICATIONS FOR FATE ASSESSMENT IN WWTPS

In this Critical Review, we reviewed existing literature on the fate of three widely consumed antibiotics (SMX, CIP, and TCY) in biological WWTPs. Factors influencing their

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elimination were assessed using the Activated Sludge Modeling framework for Xenobiotics (ASM-X) and measured removal efficiency data obtained in pilot- and full-scale WWTPs.

First, we showed that retransformation, as a result of metabolite deconjugation, can significantly impair the elimination of SMX in full-scale WWTPs. The extent of retransformation in different WWTPs was adequately predicted by ASM-X and likely explains the observed variability in SMX removal efficiencies. The combined quantification of parent and conjugated pharmaceuticals is thus required to close mass balances in WWTPs, as deconjugation is a relevant process to a number of pharmaceuticals (among others, carbamazepine, diclofenac, and sulfonamide antibiotics). Furthermore, we could determine whether retransformation is expected to prevail during secondary treatment or in upstream sewer systems. Among the responsible factors we identified the catchment size, with extensive retransformation in sewer systems of larger catchments. Different excretion patterns of SMX and its conjugated metabolites in hospitals and households were considered to explain observations in a WWTP treating hospital sewage, with negligible upstream in-sewer transport. This further indicates that substantial amounts of antibiotics administered in hospitals are not excreted to hospital sewage (but, e.g., in households), as confirmed in a recent nationwide study.[226]

The generalization of ASM-X predictions with full-scale measurements further suggested a step enhancement of SMX biotransformation kinetics undergo at SRT $\geq$ 16 d, thereby resulting in improved SMX elimination in full-scale WWTPs and in agreement with previous evidence for diclofenac.[33] Currently available experimental evidence is not sufficient to confirm this finding, and further research may be required for the quantification of transformation kinetics in treatment systems (MBRs, biofilms) operating at extended SRT.[244]

Retransformation processes were shown to be not significant for CIP fate in biological treatment systems, even though limited empirical evidence in full-scale WWTPs was available to confirm this conclusion. ASM-X predictions allowed estimating the significance of sorbed fractions in influent and effluent solids when determining CIP removal efficiency.

Using TCY as an example, we showed that dynamics in effluent loading and the presence of residual retransformable fraction can have significant implications on the assessment of the environmental risk in surface water. Conclusions as to the significance of the retransformable fraction have not yet been corroborated by empirical evidence, and further research (combining full-scale measurements and model predictions) is required to address this question for this and other pharmaceuticals.

The presented methodology is relevant to the prediction of: (i) WWTP elimination of other pharmaceuticals presenting similar characteristics to the ones assessed in this study (e.g., sulfonamides, consistently present in wastewater in acetylated form); and (ii) maximum removal efficiencies of pharmaceuticals in full-scale WWTPs, thus complementing laboratory-scale experimental studies.[245] Overall, our findings suggest the adoption of an integrated approach for the evaluation of antibiotic removal in full-scale WWTPs, where excretion and in-sewer fate processes are considered within the boundaries of the systems investigated.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.6b01899.

Details on the use of antibiotics selected in this study (S1) and on their metabolism and excretion in humans (S2), description of the activated sludge modeling framework for xenobiotics (ASM-X) (S3), overview of existing literature on the influence of SRT on the removal of pharmaceuticals in WWTPs (S4), description and examples of the model generalization methodology through the graphical comparison of measured and predicted removal efficiency (S5), metabolic pathways for sulfamethoxazole, ciprofloxacin, and tetracycline (Figures S1−S3), excreted fractions of parent and metabolites in selected human pharmacokinetic studies (Figure S4), examples of operating plots used for ASM-X generalization (Figure S5), operating plots for ciprofloxacin considering measured removal efficiency data in full-scale WWTPs and in zero-catchment scenario (Figures S6 and S7), summary of metabolism and excretion studies on sulfamethoxazole, ciprofloxacin, and tetracycline (Table S1), Gujer matrix of ASM-X (Table S2), summary of scenario simulations using ASM-X (Table S3), literature values of biotransformation and retransformation rate constants for sulfamethoxazole (Table S4), summary of literature studies reporting removal efficiency data for sulfamethoxazole and ciprofloxacin in municipal and hospital WWTPs (Tables S5 and S6), and design capacity and residence time in upstream sewer for selected WWTPs/catchments (Table S7) (PDF)

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Notes

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■ ABBREVIATIONS

ADBI	celestolide
AHTN	tonalide
ASM-X	activated sludge model framework for xenobiotics
BE	benzoyl-ecgonine
CAS	conventional activated sludge
CBZ	carbamazepine
CIP	ciprofloxacin
CIT	citalopram
COC	cocaine
CYP	cytochrome P
DCF	diclofenac
DF	dilution factor

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DZP	diazepam
EE2	17α-ethinyl estradiol
EMA	European Medicines Agency
EME	ecgonine-methyl-ester
ERA	environmental risk assessment
ERY	erythromycin
FBR	fixed bed biofilm reactor
FLX	fluoxetine
Glu	glucuronide
HHCB	galaxolide
HRT	hydraulic retention time
HYBAS	hybrid biofilm activated sludge
IBP	ibuprofen
MBBR	moving bed biofilm reactor
MBR	membrane bioreactors
MEC	measured environmental concentration
NPH	nonylphenol
NPX	naproxen
PE	population equivalent
PEC	predicted environmental concentration
PNEC	predicted no-effect concentration
REACH	Registration, Evaluation, Authorisation, and Restriction of Chemicals
ROX	roxythromycin
RQ	risk quotient
SAL	salicylic acid
SMX	sulfamethoxazole
SRT	solid retention time
TCY	tetracycline
TMP	trimethoprim
WWTP	wastewater treatment plant

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